slightly higher pressures, MACS can greatly slow down the movement of cytoplasmic molecules, likely due to increasing the volume fraction of macromolecules. Aside from being a merely biophysical observation, this phenomenon practically enables digital counting of low-abundance proteins via standard total internal reflection microscopy since the molecules diffuse marginally during a 30-milisecond exposure time and appear as diffraction limited spots. 3) Capturing rare events. Operating MACS in a mode where cells flow continuously as a single-layer enables the user to monitor up to 10 million cells per hour. On-the-fly image analysis then allows the device to instantaneously stop the flow as a cell of interest flows through, to acquire a detailed snapshot.

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Electro-Optofluidicis: Achieving Dynamic Control On-Chip

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Integrated optofluidics holds abundant promise for high throughput detection, study, and analysis of biochemical molecules and nanoparticles on chip. A vital element in integrated optofluidics is dynamic tuning and precise control of photonic devices, especially when employing electronic techniques which are challenging to utilize in an aqueous environment. We overcome this challenge by introducing a new platform in which the photonic device is controlled using electro-optical phase tuning. The phase tuning is generated by the thermo-optic effect using an on-chip electric microheater located outside the fluidic channel, and is transmitted to the optofluidic device through optical waveguides. The microheater is compact, high-speed ($>$ 18 kHz), and consumes low power ($\sim$ mW). We demonstrate dynamic optical trapping control of nanoparticles by an optofluidic resonator. This novel electro-optofluidic platform allows the realization of high throughput optofluidic devices with switching, tuning, and reconfiguration capability, and promises new directions in optofluidics.

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Real-Time, Label-Free Sensing of Epidermal Growth Factor-Induced Changes of Cell Adhesion

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Conventional approaches for assessing changes in cell adhesion often lack of time resolution and require invasive force or nonnative label. To circumvent such problems, we have developed an innovative approach of using quartz crystal microbalance with dissipation monitoring (QCM-D) to track real-time changes in cell adhesion. We have experimentally and computationally established a correlation between time-dependent changes in energy dissipation factor (ΔD) measured from the QCM-D and the level of cell adhesion complex (i.e., focal adhesions). Based on this correlation, we have been able to investigate the epidermal growth factor-induced change in cell adhesion and its regulation. We have also been able to evaluate the effects of various pharmacological interventions of this dynamic change in cell adhesion. The results of our study suggest that this QCM-D-based approach can potentially be exploited for fundamental study of cellular processes such as cell signaling, trafficking, and mechanotransduction, as well as for biomedical research on drug and biomarker screening.

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Using Hydrodynamic Forces to Trap and Study Membrane-Associated Molecules in Lipid Bilayers

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In this presentation I show how hydrodynamic forces can be used to locally trap and move membrane-associated molecules in lipid bilayers. We use the liquid flow through a ~1 μm pipette to create a localized force field that acts on molecules protruding from the lipid bilayer (see Fig. 1). In addition to introducing the hydrodynamic trap and some of the possibilities and challenges of using this technique on living cells, I will also present examples of using this technique to: (i) vary the concentration of molecules in lipid bilayers, (ii) study intermolecular interactions between different membrane-bound proteins as a function of surface coverage and (iii) induce and study pore formation in lipid bilayers. In particular, I show how the hydrodynamic trap can be used to obtain information about the orientation and mechanical properties of the extracellular domain of the tyrosine phosphatase CD45 involved in the early stages of T-cell immune response.

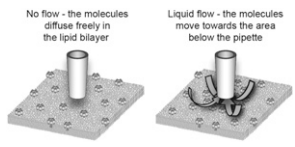

Fig. 1. Liquid flow through a conical pipette is used to locally trap molecules in lipid bilayers.

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Superresolution Inter-Surface Interaction Energy Mapping using Particle Tracking Microscopy (PTE)

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In biology, binding reactions taking place between apposing surfaces, in contrast to reactions in solution, are controlling a plethora of critical processes including cell adhesion and motility, immunological signaling, neurotransmission etc. However he techniques developed to quantitatively characterize interfacial reactions (e.g. the surface force apparatus, surface plasmon resonance and quartz crystal microbalance) are either not at all amenable to imaging or have at best a resolution of tens of micrometers. Here, we monitored the transient interaction of diffusing particles with a surface to measure quantitatively and under equilibrium conditions, inter-surface on-rates, off-rates and energies for binding reactions (1, 2). These interactions could then be laterally resolved to produce an energy map with sub-diffraction-limited resolution. This novel method was termed Particle Tracking Microscopy.

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Querying Next Generation Sequencing Data for Insight into the Ion Channel Transcriptome (Channelome) of Cultured Human Astrocytes

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In vitro culture systems are useful for investigations of cellular function and molecular interactions in a controlled environment. The experimental utility of a cell culture system is heightened by genetic characterization of the cell population under study and by knowledge of the extent to which the culture population resembles the tissue of origin. Here we report outcomes of Next Generation Sequencing (NGS) efforts to acquire a comprehensive view of the cadre of ion channels expressed in cultures of normal human astrocytes (NHA; Lonza). Experiments were undertaken with the goals of identifying novel ion channel candidates for glial function, and of assessing whether the cell culture population expressed ion channels with an established role in astrocyte function. Total RNA isolated from normal human astrocytes that were cultured for 5 days after passage was sequenced with Illumina-Solexa technology at the National Center for Genome Resources (NCGR). Approximately 23 million reads mapped to 18,470 genes on the human reference genome (HUGO nomenclature; build 37). Analysis revealed the expression of genes representative of glial and neural lineages. Genes characteristic of astrocytes were more prevalent than genes that typify oligodendrocytes. The NHA ion channel transcriptome comprised over 200 genes for voltage-gated and ligand-gated ion channels and represented 0.24% of the unique RNA Seq reads. Of particular interest were the many expected and novel genes for potassium channels (KCN-x), calcium channels (CACNA-x), and glutamate receptors (GRI-x) that were represented in the NHA channelome. Results provide a rich resource for further investigations of ion channel function, signaling pathways, and gene networks in the normal human astrocyte cell population. Supported by NIH (P50GM068762, P20RR016480).

Computational Methods I

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Coarse Grained Molecular Dynamics Simulation of the Interaction of Cytochrome C with Lipid Bilayers

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In addition to a well-characterized role of cytochrome c (cyt c) as a pro-apoptotic factor acting after its release from mitochondria into the cytosol, a new pro-apoptotic function of the intra-mitochondrial pool of cyt c has been recently identified. Early in apoptosis, cyt c interacts with a mitochondria-unique phospholipid, cardiolipin (CL), that massively transmigrates from the inner to the outer mitochondrial membrane. In the complexes thus